

**ASSESSMENT OF ANTIBACTERIAL PROPERTY OF SILVER  
COATED STAINLESS STEEL ORTHODONTIC BRACKETS  
AGAINST STREPTOCOCCUS MUTANS, LACTOBACILLUS  
ACIDOPHILUS AND PORPHYROMONAS GINGIVALIS  
– AN IN VITRO STUDY**

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**BRANCH – V**

**ORTHODONTICS AND DENTOFACIAL ORTHOPAEDICS**



**THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY  
CHENNAI – 600 032**

**2014 – 2017**

## **CERTIFICATE**

This is to certify that **Dr.D.NALINI**, Post graduate student (**2014 – 2017**) in the Department of Orthodontics and Dentofacial orthopaedics branch V, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003 has done this dissertation titled “*Assessment of antibacterial property of silver coated stainless steel orthodontic brackets against streptococcus mutans, lactobacillus acidophilus and porphyromonas gingivalis – an in vitro study*” under my direct guidance and supervision for partial fulfillment of the M.D.S degree examination in April 2017 as per the regulations laid down by The Tamil Nadu Dr. M.G.R. Medical University, Chennai -600 032 for **M.D.S., Orthodontics and Dentofacial orthopaedics (Branch – V)** degree examination.

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## **DECLARATION**

I, **Dr.D.Nalini**, do hereby declare that the dissertation titled “*Assessment of antibacterial property of silver coated stainless steel orthodontic brackets against streptococcus mutans, lactobacillus acidophilus and porphyromonas gingivalis – an in vitro study*” was done in the Department of Orthodontics, Tamil Nadu Government Dental College & Hospital, Chennai 600 003. I have utilized the facilities provided in the Government Dental College for the study in partial fulfillment of the requirements for the degree of Master of Dental Surgery in the speciality of Orthodontics and Dentofacial Orthopaedics (Branch V) during the course period **2014-2017** under the conceptualization and guidance of my dissertation guide, **Professor Dr. B. BALASHANMUGAM MDS**.

I declare that no part of the dissertation will be utilized for gaining financial assistance for research or other promotions without obtaining prior permission from The Tamil Nadu Government Dental College & Hospital.

I also declare that no part of this work will be published either in the print or electronic media except with those who have been actively involved in this dissertation work and I firmly affirm that the right to preserve or publish this work rests solely with the prior permission of the Principal, Tamil Nadu Government Dental College & Hospital, Chennai 600 003, but with the vested right that I shall be cited as the author(s).

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**Signature of the HOD**

**Signature of the Head of the Institution**

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## **TRIPARTITE AGREEMENT**

This agreement herein after the “Agreement” is entered into on this..... day of December 2016 between the Tamil Nadu Government Dental College and Hospital represented by its **Principal** having address at Tamilnadu Government Dental college and Hospital, Chennai-03, (hereafter referred to as , ‘the college’)

And

**Dr. B. BALASHANMUGAM** aged 45 years working as professor at the college, having residence address at 8-B,Crescent road, Shenoy nagar,Chennai-600030, Tamilnadu (Herein after referred to as the ‘Principal investigator’)

And

**Dr.D.Nalini** aged 32 years currently studying as postgraduate student in department of Orthodontics in Tamilnadu Government Dental College and Hospital (Herein after referred to as the ‘PG/Research student and co-investigator’).

Whereas the ‘PG/Research student as part of his curriculum undertakes to research **“Assessment of degree of treatment difficulty of maxillary canine impaction using KPG index and to analyse lateral incisor root resorption by 3D CBCT- A retrospective study”** for which purpose the PG/Principal investigator shall act as principal investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG/Research student as to the extent possible as a Co-investigator.

Whereas the parties, by this agreement have mutually agreed to the various issues including in particular the copyright and confidentiality issues that arise in this regard.

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8. It is agreed that as regards other aspects not covered under this agreement, but which pertain to the research undertaken by the student Researcher, under guidance from the Principal Investigator, the decision of the college shall be binding and final.

9. If any dispute arises as to the matters related or connected to this agreement herein, it shall be referred to arbitration in accordance with the provisions of the Arbitration and Conciliation Act, 1996.
10. In witness whereof the parties hereinabove mentioned have on this the day month and year herein above mentioned set their hands to this agreement in the presence of the following two witnesses.

College represented by its

**Principal**

**PG Student**

**Witnesses**

**Student Guide**

- 1.
- 2.

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## **ABBREVIATIONS**

<b>S.mutans</b>	-	Streptococcus mutans
<b>L.acidophilus</b>	-	Lactobacillus acidophilus
<b>P.gingivalis</b>	-	Porphyromonas gingivalis
<b>MBT</b>	-	McLaughlin Bennett Trevisi
<b>PEA</b>	-	Pre-adjusted Edgewise Appliance
<b>SS</b>	-	Stainless Steel
<b>NiTi</b>	-	Nickel Titanium
<b>CFU</b>	-	Colony Forming Unit
<b>MRS</b>	-	de Man, Rogosa and Sharpe
<b>BHI</b>	-	Brain Heart Infusion Broth
<b>MTCC</b>	-	Microbial Type Culture Collection
<b>ATCC</b>	-	America Type Culture Collection
<b>SEM</b>	-	Scanning Electron Microscope
<b>WSL</b>	-	White Spot Lesion
<b>TiO<sub>2</sub></b>	-	Titanium oxide
<b>TiAg</b>	-	Titanium silver
<b>AgBr</b>	-	Silver bromide
<b>SBS</b>	-	Shear Bond Strength
<b>NP</b>	-	Nano Particles
<b>MIC</b>	-	Minimum Inhibitory Concentrations

## **INTRODUCTION**

Fixed orthodontic treatment is the preferred and most common method for treating malocclusion. Oral environment with fixed orthodontic appliance provides conducive conditions for colonization of microorganisms as a result of their inherent morphologic irregularities. Patients have difficulty in maintaining adequate oral hygiene and the appliance provides additional sites for microorganisms to bind and colonize<sup>1</sup>. The resultant increase in oral microbial count places the patient at higher risk for enamel demineralization and periodontal disease.

The incidence of enamel demineralization after fixed orthodontic treatment can involve up to 50% of patients. The incidence of such white spot lesions around orthodontic brackets has been demonstrated within 1 month of treatment<sup>2,3</sup>. Bacterial accumulation has been detected at the 10 mm gaps at the adhesive–enamel junction<sup>4</sup>. Among different fixed orthodontic appliances, brackets could play a significant role in enamel demineralization because they are attached to the teeth throughout the entire period of orthodontic treatment. Their complex design provides a unique environment that impedes proper access to tooth surfaces for cleaning. In a study done by Eliades et al it was seen that stainless steel represented the highest critical surface tension and energy and hence can be expected to have higher plaque retaining capacity<sup>5</sup>.

Among several pathogenic organisms that accumulate and colonize in the form of plaque, *Streptococcus mutans* play a major part in initiation of white spot lesion. With established low pH, the number of lactobacilli increases and the

number of *Streptococcus mutans* decreases<sup>1,6,7</sup>. This contributes to demineralization of the teeth once the lesions are established. Preventing these lesions is an important concern for the orthodontist because these lesions are unesthetic, unhealthy and potentially irreversible.

Accumulation of dental plaque also leads to gingival inflammation<sup>8,9</sup>. However, the effect of orthodontic treatment on the periodontal tissues in the long term is questionable<sup>10</sup>. A 10-year retrospective study<sup>11</sup> concluded that orthodontic treatment during adolescence had no distinct effect upon later periodontal health. Ample evidence indicates that the gram-negative obligate anaerobe *Porphyromonas gingivalis* as a putative periodontal pathogen in subgingival dental plaque. It plays an important role in the onset and progression of periodontal disease and it is implicated as an indicator of periodontal disease<sup>12-16</sup>.

With the emergence of antibiotic-resistant strain of bacteria, certain metals particularly in nanoparticle form have attracted attention. Nanoparticles are insoluble particles having size smaller than 100 nm. Bacteria are less likely to develop resistance to metal nanoparticles as compared to conventional antibiotics<sup>17</sup>. Nanoparticles can be used either combining with dental materials or by coating the surface which aims to reduce the microbial adhesion<sup>18</sup>.

Various studies have demonstrated the effect of silver, zinc oxide, titanium oxide nanoparticles on multiple organisms<sup>18-24</sup>. Among the various metals, silver is known for its antimicrobial activity against Gram-positive and -negative bacteria, fungi, protozoa, and certain viruses, including antibiotic-resistant strains.

Because of these properties, silver is widely used in burned areas, medical devices, textile fabric, as a water purifier<sup>25</sup>. These silver nanoparticles are now being incorporated in composites, denture base resins etc for their anti microbial property<sup>25, 26</sup>. Silver coated NiTi and SS wires have also been tried for their anti microbial property<sup>27</sup>. However, silver coated orthodontic brackets have not been evaluated so far. Hence this pioneer study planned to evaluate the efficiency of silver nanoparticle coated orthodontic brackets for their anti bacterial property against *S.mutans*, *L.acidophilus*, *P.gingivalis*.

## **AIM & OBJECTIVES**

### **AIM**

To assess antibacterial property of silver coated stainless steel orthodontic brackets against *S.mutans*, *L.acidophilus*, *P.gingivalis*

### **OBJECTIVES**

- 1) To assess the antibacterial activity of silver coated stainless steel brackets against, *S.mutans*, by counting Colony Forming Units (CFU).
- 2) To assess the antibacterial activity of silver coated Stainless steel brackets against *L.acidophilus* by counting Colony Forming Units (CFU).
- 3) To assess the antibacterial activity of silver coated stainless steel brackets against *P.gingivalis* by measuring reduction in optical density
- 4) To compare the antibacterial activity of silver coated stainless steel orthodontic brackets against *S.mutans*, *L.acidophilus*.



## **REVIEW OF LITERATURE**

### **STUDIES RELATED TO PLAQUE FORMATION DURING ORTHODONTIC TREATMENT**

**Eliades et al**<sup>5</sup> (1995) conducted an in vivo study to investigate the wettability of orthodontic bracket material surfaces and the composition of salivary films adsorbed onto them after 30 and 60 minutes of intra oral exposure. Specimens from stainless steel, fiber-reinforced polycarbonate, and polycrystalline alumina bracket manufacturing raw materials were subjected to (a) contact angle measurements with a homologous series of liquids, (b) micro multiple internal reflection Fourier transform infrared spectroscopy for the characterization of the molecular composition of the in vivo adsorbed groups, and (c) incident light optical microscopy of the acquired films. The highest critical surface tension was obtained from stainless steel followed by polycarbonate and alumina, suggesting a higher potential for increased plaque-retaining capacity of the stainless steel brackets. Accordingly, the total work of adhesion and its polar and nonpolar components were consistent with the surface tension ranking. The nonpolar component of the work of adhesion was higher than its polar counterpart for all materials tested, implying a possible higher attachment prevalence for those microorganisms using dispersive forces, such as Van der Waals forces, as the predominant attachment mechanism to surfaces. Qualitative and quantitative variations were observed in the adsorbed films after 30 and 60 minutes of intraoral exposure that reflect the influence of the surface properties of these substrates on the structure of the pellicle formed in vivo.

**Menzaghi N et al**<sup>6</sup> (1991) analyzed the modifications of some components of salivary microflora (*S. mutans*, *Lactobacillus* and yeasts) induced by orthodontic treatment. He concluded that orthodontic treatment can modify the oral microflora, increasing the concentrations of cariogenic microorganisms in plaque and saliva. Their results demonstrated a high caries risk for the subjects of the experimental group; this data suggested the need for a critical evaluation of preventive protocols applied to the orthodontic patients.

**R. Chatterjee**<sup>7</sup> (1979) conducted an in vivo study to analyse effect of orthodontic band placement on the chemical composition of human incisor tooth plaque. He observed that the placement of orthodontic bands reduced the pH, calcium and phosphorus levels and increased the carbohydrate levels in the incisor plaques of 13 adolescent subjects. Maxillary plaques showed lower pH, Ca and P levels and a tendency for higher carbohydrate levels than corresponding mandibular plaques. These results were consistent with the view that pH influences plaque composition. They also provided an additional explanation concerning the trapping of food and plaque which increases dental caries susceptibility after the placement of orthodontic bands.

## **STUDIES RELATED TO GINGIVAL AND PERIODONTAL CHANGES DURING ORTHODONTIC TREATMENT**

**Zachrisson et al**<sup>28</sup> (1972) conducted a longitudinal study to assess the gingival changes during a full period of orthodontic treatment with fixed appliances. Experimental group consisted of 49 patients, 21 boys and 28 girls treated with fixed appliances in both dental arches by the edgewise technique.

Mean age was 12.5 years. The average period of treatment was 17.5 months in the maxillary arch, 19.1 months in the mandibular arch. Instructions for tooth brushing and mouth rinsing were given. Plaque accumulation and gingival status were assessed by Plaque index (PI) and Gingival index (GI) respectively, Gingival hyperplasia was recorded through linear measurements from the bottom of the clinical pockets to the gingival margin. Control group consisted of 27 boys and 26 girls with the mean age of 13.5 years. They received no orthodontic treatment, no tooth brushing or mouth rinsing instruction. The results showed that in spite of good cleaning with low PI scores, most children developed generalized moderate hyperplastic gingivitis within one to two months after the placement of the appliances. Even patients with perfect tooth cleaning developed mild inflammatory changes.

**Zachrisson et al<sup>29</sup>** in the year 1973 conducted a study to evaluate the periodontal conditions of the young individuals subjected to orthodontic treatment by the edgewise technique. Treated group consisted of 51 patients, 18 boys and 33 girls, with a mean age of 16.2 years, participants were examined approximately 2 years after removal of fixed appliances. Untreated group consisted of 54 patients, 24 boys and 30 girls with the mean age of 16.3 years. Pocket depth, attachment loss, crown height were measured. Results showed that orthodontic patients demonstrated slightly but significantly more loss of attachment clinically than did the reference subjects. Mean loss of attachment was 0.41mm in the orthodontic group and 0.11mm in the reference group but the individual variation was large. Paired comparison for corresponding tooth surfaces in the treated and untreated groups revealed consistently higher figures for loss of attachment in the orthodontic patients.

**Kloehn JS et al<sup>30</sup>** (1974) studied the response of the periodontium during and following orthodontic treatment in 50 patients. The edgewise appliance was used for treatment of all patients prior to beginning of treatment at three month intervals during treatment and four months after treatment was completed, each patient was given a periodontal examination consisting of measurement of gingival sulcus, measurement of length of clinical crowns of twelve teeth, determination of Russel's periodontal index, determination of oral debris as described by Green and Vermillion. They concluded that inflammatory and hyperplastic changes in gingiva occurred during treatment were reversible upon appliance removal and periodontium was in better health following treatment. Orthodontic treatment did not cause any irreversible periodontal destruction. There was a direct relationship between oral hygiene and periodontal disease.

**Bollen M et al<sup>9</sup>** (2008) reviewed the effects of orthodontic therapy on periodontal health. The authors completed electronic searches in eight databases (1980-2006) and hand searches in six dental journals (1980-2006). They extracted data using standardized forms and calculated weighted mean differences. Results showed that weak evidence from one randomized study and 11 nonrandomized studies suggested that orthodontic therapy was associated with 0.03 millimeters of gingival recession, 0.13 mm of alveolar bone loss and 0.23mm of increased pocket depth when compared with no treatment. The effects of orthodontic therapy on gingivitis and attachment loss were inconsistent. They identified an absence of reliable evidence describing positive effects of orthodontic treatment on periodontal health. The existing evidence suggests that orthodontic therapy results in small detrimental effects to the periodontium.

**Aass AM et al<sup>10</sup>** (1988) conducted a radiographic study to assess the prevalence of radiographic alveolar bone loss, as related to selected background variables in 2767 14-year old schoolchildren. Bone loss was recorded when the distance from the cemento-enamel junction to the alveolar crest exceeded 2 mm. The radiographs were magnified approximately 10 times. 3% of the subjects and 18.5% of the sites were excluded because of indistinct radiographic reference points. Radiographic bone loss was found in 4.5% of the subjects. Horizontal lesions were more prevalent than vertical defects. Most subjects with bone loss had 1 (75%) or 2 (22%) lesions. No subject was diagnosed with juvenile periodontitis kind of lesion. The prevalence of bone loss depended on the variables sex, orthodontic treatment and ethnic background.

**A.M. Polson et al<sup>11</sup>** (1988) studied Long-term periodontal status after orthodontic treatment. This study evaluated the clinical periodontal status of persons who had completed orthodontic therapy at least 10 years previously (study) and compared the findings to those of adults with untreated malocclusions (control). Subjects in the study (n = 112; 63 female subjects, 49 male subjects; mean age  $29.3 \pm 4.2$  [SD] years) and control (n = 111; 62 female subjects, 49 male subjects; mean age  $32.9 \pm 6.5$  years) populations underwent a comprehensive periodontal examination that consisted of measurements taken at six points around the circumference of each tooth: (1) plaque, (2) visual inflammation, (3) bleeding after probing, (4) pocket depth, (5) gingival recession, and (6) loss of connective tissue attachment. The results showed that differences in age distribution within the groups were affecting the comparisons between the groups. Consequently, the groups were balanced for age and analyses were done to investigate group

differences by means of multiple regression techniques. The comparisons showed no significant differences between the groups for any of the periodontal variables. It was concluded that orthodontic treatment during adolescence had no discernible effect upon later periodontal health.

## **STUDIES RELATED TO WHITE SPOT LESION IN ORTHODONTICS**

**Leonard Gorelick<sup>2</sup>** (1982) conducted a study on incidence of white spot formation after bonding and banding. To establish a base line of comparison, the presence of white spots in a random sample of untreated persons were observed. The incidence of white spots among patients treated by a multi bonded technique was recorded at the time of bonding. It was found that individual teeth, banded or bonded, exhibited significantly more white spot formation than was found in the control group.

**Jon Årtun et al<sup>3</sup>** (1986) conducted a study to examine the prevalence, localization and distribution of carious white spots on vestibular tooth surfaces after orthodontic treatment with multi bonded appliances. Two test groups (A and B) were established, each comprising 60 consecutively treated adolescents from two different orthodontic practices, and a reference group of 60 persons, representing pupils from three local school classes who had not received orthodontic treatment. No differences in distribution of gender were observed between the groups. The patients in groups A and B were examined 1.8 and 1.0 years after removal of bonded appliances, respectively. Prior to treatment, patients in groups A and B were encouraged and instructed in the practice of oral hygiene and given a prescription of sodium fluoride for daily rinsing. The fluoride

programme was monitored more closely and encouragement was given more frequently in group A than in group B. Carious white spots were scored on a scale from 1 to 3 according to opacity and extension on vestibular enamel surface areas outside the area covered by bracket and bonding material during treatment. The results demonstrated significantly higher scores both for opacity and extension of the lesions in group B than in the reference group ( $P < 0.01$ ). No significant differences were observed between group A and the reference group and between groups A and B. The majority of the lesions were scored in gingival areas, and especially affected teeth were maxillary lateral incisors and mandibular canines and premolars.

**Warat sukontapatipark et al<sup>4</sup>** (2001) conducted a scanning electron microscopy study to assess the bacterial plaque accumulation adjacent to the orthodontic brackets. Experiments were carried out on 11 subjects who were scheduled for orthodontic treatment including two or four premolars. Metal brackets were bonded to the premolars to be extracted using macrofilled bonding composite. A conventional elastomeric ring was placed around one bracket and steel ligature wire around the bracket on contra lateral tooth. The subjects were told to continue their normal oral hygiene regimen. Teeth were extracted at 1, 2 or 3 weeks, after bracket bonding. Scanning electron microscopy of brackets, excess composite, and buccal enamel revealed mature plaque was present on the excess composite at 2 to 3 weeks after bonding, whereas plaque on gingival enamel surface was still at early stage of development. Results demonstrated that excess composite around the bracket base is critical site for plaque accumulation due to its rough surface and presence of distinct gap at the composite and enamel

interface. The method of ligation does not appear to influence the bacterial morphotypes on both composite and enamel surfaces.

**Höchli D et al**<sup>31</sup> (2016) reviewed the therapeutic and adverse effects of interventions to treat post-orthodontic WSLs from randomized trials in human patients. An unrestricted electronic search of eight databases from inception to May 2016 was taken. Randomized controlled trials assessing any interventions for post-orthodontic WSLs on human patients were selected. After duplicate study selection, data extraction, and risk of bias assessment according to the Cochrane guidelines, random-effects meta-analyses of mean differences (MDs), standardized mean differences (SMDs), and odds ratios (ORs), including their 95% confidence intervals (CIs) were performed, followed by subgroup and sensitivity analyses. Results showed that a total of 20 unique studies and a total of 942 patients were included, with an average age of 16.2 years and a mean number of 8.2 WSLs per patient. These were allocated to adjunct treatment with casein phosphopeptide-stabilized amorphous calcium phosphate creams, external tooth bleaching, low- or high-concentration fluoride films, gels, mouth rinses or varnishes, resin infiltration, miswak chewing sticks, bioactive glass tooth paste, or to no adjunct treatment (i.e. conventional oral hygiene). The monthly use of fluoride varnish was the best supplement to improve WSLs in terms of lesion area and enamel fluorescence followed by the use of fluoride film. WSL treatment did not provide a considerable improvement in their clinical evaluation, with imprecision due to small sample size being the main limitation of existing evidence. They concluded that based on the existing trials, interventions for post-



orthodontic WSLs, mainly fluoride varnish, seem to be effective, but further research is needed to elucidate their clinical relevance.

## **STUDIES RELATED TO ANTIBACTERIAL ACTIVITY OF NANOPARTICLES**

**Yamamoto K et al**<sup>32</sup> (1996) conducted an in vivo investigation on Antibacterial activity of silver ions implanted in SiO<sub>2</sub> filler on oral streptococci. SiO<sub>2</sub> filler samples (0.1g) were implanted with silver ions. The effect of the filler with silver ions (Ag<sup>+</sup> filler) was tested on oral streptococci bacteria. These bacterial strains had been isolated predominantly from composite resin surfaces. The organisms tested were anaerobically cultured in 5 mL Trypticase Soy Broth containing 0.5 per cent yeast extract at 37 degrees C for 10-12 h. Each bacterial strain was adjusted to a concentration of  $1 \times 10^6$  cells per mL with reduced transport fluid. Ag<sup>+</sup> filler was immersed in 1 mL of RTF and anaerobically incubated 2, 6 and 12 h to study the antibacterial effect. The survival of bacteria was then estimated by culturing on TSBY agar plates. A plate with approximately 100 discrete colonies was chosen from the serial agar cultures, and the number of colonies was counted at each sampling time. Results showed that The Ag<sup>+</sup> filler showed significantly more antibacterial activity than the control filler without silver ions. These results indicate that the antibacterial effect found in this study was due to the silver ions released by the Ag<sup>+</sup> filler and that it may be useful to add this filler to composite resin dental materials for secondary caries protection.

**Mi-Jin Chun et al**<sup>33</sup> (2007) carried out surface modification of orthodontic wires with photo catalytic titanium oxide for its antiaherent and

antibacterial properties against *S. mutans*. Stainless steel orthodontic wires were coated with  $\text{TiO}_2$  by sol gel dip coating method. Bacterial adhesion to the wires was evaluated by the weight change of wires. The antibacterial activity of surface modified orthodontic wires was demonstrated by the dilution agar plate method for *S. mutans*. The study revealed that orthodontic wires coated with  $\text{TiO}_2$  showed an antiadherent effect against *S. mutans*, compared with the uncoated wires. The bacterial mass that bound to  $\text{TiO}_2$  - coated orthodontic wires remain unchanged whereas that of uncoated wires increased by 4.97%. The  $\text{TiO}_2$ - coated wires had bactericidal action on *S. mutans*. This study concluded that the surface modification of orthodontic wires with photo catalytic  $\text{TiO}_2$  could be used with the added advantage of preventing the development of dental plaque during orthodontic treatment.

**Hannig M et al** <sup>34</sup> (2007) conducted a study was to investigate the effect of an experimental, low surface free energy nano-composite coating material on biofilm formation in situ. For this purpose, an organic/inorganic nano-composite coating with a surface free energy of 18-20 mJ/m<sup>2</sup> was applied to enamel as well as titanium specimens. The nano-composite coated specimens and un-coated controls were attached to removable intraoral splints and carried by volunteers over 24 h in the oral cavity. After intraoral exposure, specimens were processed for transmission electron microscopic analysis. On non-coated enamel and titanium control samples a multi-layer of adherent bacteria was found. In contrast, on nano-composite coated specimens strongly reduced biofilm formation was observed. In most areas of the surface-coated specimens only a 10-20 nm thick electron dense layer of adsorbed salivary proteins with adherent

protein agglomerates of 20-80 nm diameter could be detected. In addition, detachment of the adsorbed biofilm from the nano-composite coated surfaces was evident in electron microscopic micrographs. This investigation provides ultra structural evidence that it is possible to cover enamel as well as titanium with a nano-composite coating revealing easy-to-clean surface properties that cause reduced biofilm formation and accelerated removal of adherent biofilms under oral conditions.

**Bishara SE et al<sup>35</sup>** in the year 2007 conducted a study to compare the shear bond strength of a nano-hybrid restorative material, Grandio (Voco, Cuxhaven, Germany), to that of a traditional adhesive material (Transbond XT; 3M Unitek,) when bonding orthodontic brackets. Forty teeth were randomly divided into 2 groups: 20 teeth were bonded with the Transbond adhesive system and the other 20 teeth with the Grandio restorative system, following manufacturer's instructions. Student t test was used to compare the shear bond strength of the 2 systems. Significance was predetermined at  $P = 0.05$ . The t test comparisons ( $t = 0.55$ ) of the shear bond strength between the 2 adhesives indicated the absence of a significant ( $P = 0.585$ ) difference. The mean shear bond strength for Grandio was  $4.1 \pm 2.6$  MPa and that for Transbond XT was  $4.6 \pm 3.2$  MPa. During debonding, 3 of 20 brackets (15%) bonded with Grandio failed without registering any force on the Zwick recording. None of the brackets bonded with Transbond XT had a similar failure mode. They concluded that the newly introduced nano-filled composite materials can potentially be used to bond orthodontic brackets to teeth if its consistency can be more flowable to readily adhere to the bracket base.

**Redlich M et al<sup>36</sup>** (2008) conducted a study to evaluate friction between orthodontic stainless wires and bracket by coating the wire with nickel-phosphorous electroless film impregnated with inorganic fullerene-like nanoparticles of tungsten disulfide (IF-WS(2)) which are potent dry lubricants. Coating was performed by inserting stainless steel (SS) wires into electroless solutions of nickel-phosphorus (Ni-P) and IF-WS(2). The coated wires were analyzed by SEM (scanning electron microscope) and Energy Dispersive X-ray Spectrometer as well as by tribological tests using a ball-on-flat device. Friction tests simulating arch wire functioning of the coated and uncoated wires were carried out by an Instron machine. The adhesion properties of the coated wires after friction were analyzed by a Raman microscope. Results showed that SEM/EDS analysis of the coated wires showed clear impregnation of the IF-WS(2) nanoparticles in the Ni-P matrix. The friction coefficient measured by the ball-on-flat tribometer was significantly reduced (from 0.25 to 0.08). The friction forces as measured with the Instron on the coated wire were reduced by up to 54. Raman spectra showed that even after extensive friction tests the Ni-P with the IF-WS) nanoparticles is attached to the underlying stainless steel wire. It is proposed that the wires coated with these nanoparticles might offer a novel opportunity to substantially reduce friction during tooth movement. A few tests undertaken to evaluate the toxicity of the fullerene-like nanoparticles have provided indications that they might be biocompatible.

**Woo Kyung Jung et al<sup>37</sup>** (2008) conducted a study to test antibacterial effect and mechanism of action of a silver ion solution that was electrically generated were investigated for *Staphylococcus aureus* and *Escherichia coli* by

analysing the growth, morphology, and ultra structure of the bacterial cells following treatment with the silver ion solution. Bacteria were exposed to the silver ion solution for various lengths of time, and the antibacterial effect of the solution was tested using the conventional plate count method and flow cytometric (FC) analysis. Reductions of more than 5 log<sub>10</sub> CFU/ml of both *S. aureus* and *E. coli* bacteria were confirmed after 90 min of treatment with the silver ion solution. Significant reduction of *S. aureus* and *E. coli* cells was also observed by FC analysis; however, the reduction rate determined by FC analysis was less than that determined by the conventional plate count method. Transmission electron microscopy showed considerable changes in the bacterial cell membranes upon silver ion treatment, which might be the cause or consequence of cell death. This study concluded that silver ions may cause *S. aureus* and *E. coli* bacteria to reach an active but nonculturable state and eventually die.

**Jung-Yoon Choi et al<sup>38</sup>** (2009) Conducted a study to test Photo catalytic Antibacterial Effect of TiO<sub>2</sub> Film of TiAg on *S. mutans*. Photo catalytic antibacterial effects on *S. mutans* of Ti and TiAg substrates coated with two crystalline forms of TiO<sub>2</sub> by thermal and anodic oxidation. A bacterial suspension of *S. mutans* was applied onto TiO<sub>2</sub>-coated metal specimens and uncoated specimens with ultraviolet A (UVA) illumination for 20 to 100 minutes. The same specimen without UVA was used as the control. The level of colony-forming units of *S. mutans* after UVA illumination was compared with that of the control. The study concluded that TiO<sub>2</sub> coating on TiAg had a significantly higher and more rapid antibacterial effect than did the TiO<sub>2</sub> coating on Ti. The addition of Ag to

the Ti specimen indicated a synergistic effect on the photo catalytic antibacterial property against S mutans.

**Ahn SJ et al<sup>39</sup>** (2009) conducted a study to compare experimental composite adhesives containing silica nanofillers and silver nanoparticles with two conventional adhesives (composite and resin-modified glass ionomer [RMGI]) to analyze surface characteristics, physical properties and antibacterial activities against cariogenic streptococci. Surface roughness and surface free energy (SFE) characteristics were measured using confocal laser scanning microscopy and the sessile drop method. Shear bond strength and bond failure interface were analyzed to compare the physical properties. Antimicrobial activities were analyzed by a bacterial adhesion assay, a disk diffusion test, and an optical density measurement of bacterial suspension containing each adhesive. Results showed that ECAs had rougher surfaces than conventional adhesives due to the addition of silver nanoparticles. ECAs had more similar SFE characteristics to composite than to RMGI. Bacterial adhesion to ECAs was less than to conventional adhesives, which was not influenced by saliva coating. Bacterial suspension containing ECAs showed slower bacterial growth than those containing conventional adhesives. There was no significant difference in shear bond strength and bond failure interface between ECAs and conventional adhesives.

**Tancan Uysal et al<sup>40</sup>** (2010) conducted a study was to test nano-composite (Filtek Supreme Plus Universal) and a newly introduced nano-ionomer (Ketac™ N100 Light Curing Nano-Ionomer) restorative to determine their shear

bond strength (SBS) and failure site locations in comparison with a conventional light-cure orthodontic bonding adhesive (Transbond XT). Sixty freshly extracted human maxillary premolar teeth were arbitrarily divided into three equal groups. The brackets were bonded to the teeth in each group with different composites, according to the manufacturers' instructions. The SBS values of the brackets were recorded in Megapascals (MPa) using a universal testing machine. Adhesive remnant index scores (ARI) were determined after failure of the brackets. The data were analysed using analysis of variance, Tukey honestly significant difference, and chi-square tests. The results demonstrated that group 1 (Transbond XT) had a higher SBS than that of group 2 (nano-composite, mean:) and group 3 (nano-ionomer,). No significant differences in debond locations were found among the three groups. They concluded that nano-composites and nano-ionomers may be suitable for bonding since they fulfil the previously suggested SBS ranges for clinical acceptability, but they are inferior to a conventional orthodontic composite.

**Liao Juan et al<sup>41</sup>** (2010) studied antibacterial effectiveness of silver nanoparticle coated implanted devices. They prepared a silver nanoparticle-modified titanium (Ti-nAg) surface using silanization method. The morphology and chemical components of the Ti-nAg surface were characterized by scanning electron microscopy (SEM) equipped with energy-dispersive spectroscopy (EDS). Two species of bacteria, *Staphylococcus aureus* and *Escherichia coli*, were utilized to test the antibacterial effect of the Ti-nAg treated surface. The SEM examination revealed that a small quantity of silver nanoparticles was sparsely deposited on the titanium surface. The diameter of these nanoparticles ranged

from ten to several hundred nm. EDS analyses revealed that there was 4.26% of Ag present on the surface. After a 24-hour incubation, 94% of *Staphylococcus aureus* and over 95% of *Escherichia coli* had been killed on the Ti-nAg surface, and the SEM examination of anti-adhesive efficacy test showed that there were less bacteria attached to Ti-nAg surface than to a control surface of untreated Titanium. They concluded that silver nanoparticle-modified titanium is a promising material with an antibacterial property that may be used as an implantable biomaterial.

**Alok Girish Shah et al<sup>42</sup>** (2011) carried out an in vitro study on the antibacterial and anti adherent effect of Titanium oxide surface modified stainless steel wires against the *Lactobacillus Acidophilus* bacterial strain. This study was done on 120 specimens of stainless steel pre adjusted Edgewise appliance (PEA) orthodontic brackets. The specimens were divided into four test groups. Groups containing uncoated brackets acted as a control group for their respective experimental group containing coated brackets. Surface modification of brackets was carried out by the radiofrequency magnetron sputtering method with photo catalytic TiO<sub>2</sub>. Brackets then were subjected to microbiological tests for assessment of the antiadherent and antibacterial properties of photo catalytic TiO<sub>2</sub> coating against *Lactobacillus acidophilus*. Results showed that Orthodontic brackets coated with photo catalytic TiO<sub>2</sub> showed an anti adherent effect against *L. acidophilus* compared with uncoated brackets. Control group had an initial average weight of  $0.0722 \pm 0.0009$  and a final average weight of  $0.0741 \pm 0.0018$  with an increase in weight of 0.0019 (2.6%), which was statistically significant. A 4.1% weight change in uncoated brackets was noted compared with a 2.6%



change in surface modified brackets (0.0029 vs 0.0019 weight change). The bacterial mass that was bound to the TiO<sub>2</sub>-coated brackets was less when compared with the uncoated brackets. The survival rate of bacterial cells was calculated in terms of CFUs. the survival rate of lactobacilli was  $80.50 \pm 12.88$  CFUs in the case of control group, whereas it was  $52.20 \pm 12.79$  CFUs in the case of experimental group . Antibacterial activity in CFUs was statistically less significant in experimental group than in control group. They concluded that Surface modification of orthodontic brackets with photo catalytic TiO<sub>2</sub> can be used to prevent the accumulation of dental plaque and the development of dental caries during orthodontic treatment.

**Laura Susana Acosta et al<sup>26</sup>** (2012) studied the Cytocompatibility of antifungal acrylic resin containing silver nanoparticles for dentures in preventing the development of denture stomatitis. The safety of a new dental material with antifungal properties was analyzed in this work. Poly methyl methacrylate [PMMA] discs and PMMA-silver nano particle discs were formulated, with the commercial acrylic resin, Nature-Cryl<sup>TM</sup>, used as a control. Silver nanoparticles were synthesized and characterized by ultraviolet-visible spectroscopy, dispersive Raman spectroscopy, and transmission electron microscopy. The antifungal effect was assessed using a luminescent microbial cell viability assay. Biocompatibility tests were carried out using NIH-3T3 mouse embryonic fibroblasts and a Jurkat human lymphocyte cell line. Cells were cultured for 24 or 72 hours in the presence or absence of the polymer formulations and analyzed using three different tests, ie, cellular viability by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, and cell proliferation by enzyme-

linked immunosorbent assay BrdU, and genomic DNA damage (Comet assay). Finally, the samples were evaluated mechanically, and the polymer-bearing silver nanoparticles were analyzed microscopically to evaluate dispersion of the nanoparticles. Results showed that PMMA-silver nanoparticle discs significantly reduce adherence of *C. albicans* and do not affect metabolism or proliferation. They concluded that PMMA silver nanoparticle is not genotoxic and denture base material containing silver nanoparticles is biocompatible

**Maryam Poosti et al<sup>43</sup>** (2013) studied shear bond strength (SBS) and antibacterial effects of an orthodontic composite after adding titanium oxide nanoparticles. Light cure orthodontic composite paste was blended with TiO<sub>2</sub> nanoparticles. A total of 30 extracted premolars were randomly allocated into two groups of 15. In order to bond brackets, Transbond XT adhesive and nanocomposite were used in each group, respectively. SBS of two groups were determined, and the adhesive remnant index scores were assessed. A total of 45 composite discs specimen were prepared. Of the 45 discs, 30 discs were made from nanocomposite and tested for antibacterial properties immediately and 30 days after curing by direct contact test. The antibacterial properties of the remaining 15 discs that were made from the conventional composite were tested immediately after curing as control group. Student's t-test and chi-square tests were used to analyze the data with the significance level of 0.05. No significant difference was found between SBS of conventional and nanocomposites. ARI (Adhesive Remnant Index) scores of two groups were not significantly different after debonding. Comparison of antibacterial effects between conventional and nanocomposite demonstrated significant difference between two groups, with

nanocomposites having a higher antibacterial activity. Colony count revealed no significant difference in bacterial growth immediately and 30 days after curing in nanocomposite group. They concluded that adding TiO<sub>2</sub> nanoparticles to orthodontic composite enhances its antibacterial effects without compromising the SBS.

**Azarsina et al<sup>44</sup>** (2013) studied the antibacterial properties of composite resin containing nanosilver against *S.mutans* and *Lactobacillus*. In this study Nanosilver was added to Z250 composite at 0.5 and 1% by weight. In order to confirm the homogenous distribution of the nanoparticles in the composite resin, SEM-EDX analysis was performed on one sample in each group. Z250 composite without nanosilver was used as control. Direct contact test was used to test the antibacterial properties of nanoparticle-loaded composites: 0.001 ml of 0.5 Mc Farland suspension of MS and L was placed on composite disks, and incubated for 1 hour in 5 to 10% CO<sub>2</sub> incubator at 37°C. Samples were placed in 0.5 ml of sterile BHI broth and incubated for 2 hours in CO<sub>2</sub> incubator. Afterwards, 0.001 ml liquid from each medium was distributed on blood agar plates and incubated for 48 hours in CO<sub>2</sub> incubator. The numbers of bacterial colonies were counted visually. Results showed that Addition of nanosilver to composite resin had a significant effect on reduction of the number of *S.mutans* and *Lactobacillus* colonies. The antibacterial properties of composite resins are different depending on the concentration of nanosilver. Tukey test indicated that increase in the concentration of nanosilver caused the increase in antibacterial properties of composite resin. They concluded that Addition of silver nanoparticles to Z250 composite could significantly inhibit the

growth of *Streptococcus mutans* and *Lactobacillus* on the surface of this composite and therefore prevent the occurrence of secondary caries.

**Azam akhavan et al<sup>45</sup>** in the year 2013 conducted a study to investigate the effect of incorporating Silver and Hydroxyapatite (HA) nanoparticles on the shear bond strength (SBS) of an orthodontic adhesive. Silver and HA nanoparticles were prepared by gamma irradiation and inspected by scanning electron microscopy and EDAX analysis. The nanoparticles were added to the primer of Transbond XT in 1%, 5% and 10% silver concentrations. Each compound (along with a control) was used for bonding stainless steel brackets to 12 human premolars (48 in total) and the SBS of all samples, along with their ARI scores were measured. Results showed that the SBS of the control, 1%, 5% and 10% nanoparticle groups were  $12.06 \pm 5.48$ ,  $20.66 \pm 5.72$ ,  $10.77 \pm 8.16$  and  $5.40 \pm 2.00$  MPa, respectively. A significant difference existed between all study groups, except for the control–5% and 5%–10% study groups. There was no statistically significant difference in distribution of ARI scores across the study groups ( $p = 0.44$ ). It was concluded that the Incorporation of silver/HA nanoparticles containing 5% and 1% silver maintains and increases the SBS of orthodontic adhesives, respectively, whereas increasing the amount of particles to 10% has an undesirable effect when compared to the control group.

**Tianlu Zhanget al<sup>46</sup>** in the year 2014 studied Cytotoxic Potential of Silver Nanoparticles. Silver nanoparticles (AgNPs) have been widely used in industrial, household, and healthcare-related products due to their excellent antimicrobial activity. With increased exposure of AgNPs to human beings, the risk of safety

has attracted much attention from the public and scientists. The potential impact of AgNPs on individuals was studied at the cell level. Main effects mediated by AgNPs on the cell, such as cell uptake and intracellular distribution, cytotoxicity, genotoxicity, and immunological responses, as well as some of the major factors that influence these effects *in vitro* and *in vivo*, such as dose, time, size, shape, surface chemistry, and cell type. At the end, the main influences on the cell and indicate the challenges in this field, which are helpful for assessing the risk of AgNPs in future were summarized.

**Sarika Chhattani et al<sup>47</sup>** in the year 2014 carried out surface modification of stainless steel and Nickel Titanium coated orthodontic wires with photo catalytic titanium oxide for its ant adherent and antibacterial properties against *S.mutans*. Surface modification of both stainless steel and nickel titanium orthodontic wires with photo catalytic titanium oxide was carried out by sol gel thin film dip coating method. Bacterial adhesion to the wires was evaluated by the weight change of wires. The antibacterial activity of surface modified orthodontic wires was demonstrated by the dilution agar plate method for *S.mutans*. The study revealed that orthodontic wires coated with  $\text{TiO}_2$  showed an anti adherent effect against *S.mutans*, compared with the uncoated wires. Uncoated stainless steel wires showed 35.4% increase in weight, whereas surface modified wires showed only 4.08% increase in weight. It can also be seen that uncoated nickel titanium wires showed 20.5% increase in weight, whereas surface modified wires showed only 4.4% increase in weight. Thus, uncoated orthodontic wires showed statistically significant increase in the weight when compared with the surface modified orthodontic wires. The bacterial mass that bound to  $\text{TiO}_2$  - coated

orthodontic wires remain unchanged. The study concluded that the surface modification of orthodontic wires with photo catalytic TiO<sub>2</sub> could be used with the added advantage of preventing the development of dental plaque during orthodontic treatment.

**Shahin Kasraei et al**<sup>48</sup> in the year 2014-studied the antibacterial properties of composite resins containing 1% silver and zinc-oxide nanoparticles on *S. mutans* and *Lactobacillus*. Ninety discoid tablets containing 0%, 1% nano-silver and 1% nano zinc-oxide particles were prepared from flowable composite resin (n = 30). The antibacterial properties of composite resin discs were evaluated by direct contact test. Diluted solutions of *Streptococcus mutans* (PTCC 1683) and *Lactobacillus* (PTCC 1643) were prepared. 0.01 ml of each bacterial species was separately placed on the discs. The discs were transferred to liquid culture media and were incubated at 37°C for 8 hr. 0.01 ml of each solution was cultured on blood agar and the colonies were counted. Results showed that Composites containing nano zinc oxide particles or silver nanoparticles exhibited higher antibacterial activity against *S. mutans* and *Lactobacillus* compared to the control group. The effect of zinc-oxide on *S. mutans* was significantly higher than that of silver. There were no significant differences in the antibacterial activity against *Lactobacillus* between composites containing silver nanoparticles and those containing zinc-oxide nanoparticles. They concluded that Composite resins containing silver or zinc oxide nanoparticles exhibited antibacterial activity against *S. mutans* and *Lactobacillus*.

**Alexandros Besinis**<sup>49</sup> et al (2014) investigated the toxicity of silver, titanium dioxide and silica nanoparticles against the oral pathogenic species of *S. mutans*,

compared to the routine disinfectant, chlorhexidine. The bacteria were assessed using the minimum inhibitory concentration assay for growth, fluorescent staining for live/dead cells, and measurements of lactate. All the assays showed that Ag NPs had the strongest antibacterial activity of the NPs tested, with bacterial growth also being 25-fold lower than that in chlorhexidine. The survival rate of bacteria under the effect of 100 mg l Ag NPs in the media was 2% compared to 60% with chlorhexidine, while the lactate concentration was 0.6 and 4.0 mM, respectively. Silica and titanium dioxide NPs had limited effects. Dialysis experiments showed negligible silver dissolution. Overall, Ag NPs were the best disinfectant and performed better than chlorhexidine.

**Ronghe Zhang et al<sup>50</sup>** in the year 2015 studied antibacterial property and biocompatibility of nano Ag/TiO<sub>2</sub> coating bracket for the common bacteria in oral cavity. Ag/TiO<sub>2</sub> thin film was used for coating on the surface of ordinary metal bracket with spin-on deposition to prepare nano Ag/TiO<sub>2</sub> coating bracket. Micro morphology in the surface of nano Ag/TiO<sub>2</sub> coating bracket was detected by scanning electron microscope (SEM), and surface roughness of ordinary mental bracket, nano TiO<sub>2</sub> coating bracket and nano Ag/TiO<sub>2</sub> coating bracket were measured. First, antibacterial property of nano Ag/TiO<sub>2</sub> coating bracket on the common bacteria in oral cavity was studied by sticking membrane method. Secondly, bonding strength of nano TiO<sub>2</sub> coating and nano Ag/TiO<sub>2</sub> coating bracket in groups were detected by scratching test. The result showed that, the synthetic nano Ag/TiO<sub>2</sub> coating was nanogranular films with rigorous organizational structure, presenting as smooth and clean surface, and antibacterial rate of nano Ag/TiO<sub>2</sub> coating for the common bacteria in oral cavity for 20 min

was more than 79% in the dark. All the findings suggested that, nano Ag/TiO<sub>2</sub> coating bracket not only has antibacterial effect but also has good biocompatibility, therefore, it can satisfy the clinical request of orthodontic treatment.

**A. Jesline et al<sup>51</sup>** in the year 2015 studied the efficacy of zinc and titanium dioxide nanoparticles against biofilm producing methicillin-resistant *Staphylococcus aureus*. Biofilm production was detected by tissue culture plate method. Out of 30 MRSA isolates, 22 isolates showed strong biofilm production and 2 showed weak and moderate biofilm formation. Two strong and weak biofilm-producing methicillin resistant *S. aureus* isolates were subjected to antimicrobial activity using commercially available zinc and titanium dioxide nanoparticles. Thus, the nanoparticles showed considerably good activity against the isolates, and it can be concluded that they may act as promising, antibacterial agents in the coming years.

**Petr Suchomel et al<sup>52</sup>** in the year 2015 conducted a Comparative Study of Antimicrobial Activity of AgBr and Ag Nanoparticles (NPs). The diverse mechanism of antimicrobial activity of Ag and AgBr nanoparticles against gram-positive and gram-negative bacteria and also against several strains of candida was explored in this study. The AgBr nanoparticles (NPs) were prepared by simple precipitation of silver nitrate by potassium bromide in the presence of stabilizing polymers. The used polymers influence significantly the size of the prepared AgBr NPs dependently on the mode of interaction of polymer with Ag<sup>+</sup> ions. Small NPs (diameter of about 60–70 nm) were formed in the presence of the



polymer with low interaction as are PEG and HEC, the polymers which interact with  $\text{Ag}^+$  strongly produce nearly two times bigger NPs (120–130 nm). The prepared AgBr NPs were transformed to Ag NPs by the reduction using  $\text{NaBH}_4$ . The sizes of the produced Ag NPs followed the same trends – the smallest NPs were produced in the presence of PEG and HEC polymers. Antibacterial and antifungal efficacies of the prepared AgBr and Ag NPs were tested using the standard dilution method which enables to determine the minimum inhibitory concentrations (MICs) of the tested samples necessary to inhibit the growth of the bacterial strains and yeasts. The obtained results of antimicrobial activity of AgBr and Ag NPs were discussed in terms of possible mechanism of the action of these NPs against tested microbial strains. The AgBr NPs were more effective against gram-negative bacteria and tested yeast strains while Ag NPs showed the best antibacterial action against gram-positive bacteria strains. The realized antibacterial assays showed differences between antimicrobial action of AgBr and Ag nanoparticles and  $\text{Ag}^+$  ions used as reference material. The differences were connected to the interaction of the silver based materials with the cell walls of microorganisms. It was clearly proved that from the studied silver materials the gram-positive bacteria strains are most sensitive to Ag NPs probably due to destructive interaction of these NPs with the cell walls. On the other hand, AgBr NPs are more efficient against gram-negative bacteria and yeast microbial strains due to the combination of interaction of solid AgBr nanoparticles with cell walls and simultaneous penetration of  $\text{Ag}^+$  ions into the cells. This observation of different antimicrobial activity of various forms of NPs based on the metallic silver or silver bromide respectively could be the guideline for the future

formulations of the antimicrobials targeted to have a specific mode of action against different types of microbial strains.

**Arun Rameshwar Mhaske et al<sup>27</sup>** in the year 2015 conducted in vitro study is to assess the antiadherent and antibacterial properties of surface-modified stainless steel and NiTi orthodontic wires with silver against *L. acidophilus*. This study was done on 80 specimens of stainless steel and NiTi orthodontic wires. The specimens were divided into eight test groups. Each group consisted of 10 specimens. Groups containing uncoated wires acted as a control group for their respective experimental group containing coated wires. Surface modification of wires was carried out by the thermal vacuum evaporation method with silver. Wires were then subjected to microbiological tests for assessment of the antiadherent and antibacterial properties of silver coating against *L. acidophilus*. Mann–Whitney U test was used to analyze the colony-forming units (CFUs) in control and test groups; and Student's t test (two-tailed, dependent) was used to find the significance of study parameters on a continuous scale within each group. Results showed that Orthodontic wires coated with silver showed an antiadherent effect against *L. acidophilus* compared with uncoated wires. Uncoated stainless steel and NiTi wires respectively showed 35.4 and 20.5 % increase in weight which was statistically significant, whereas surface-modified wires showed only 4.08 and 4.4 % increase in weight which was not statistically significant. The groups containing surface-modified wires showed statistically significant decrease in the survival rate of *L. acidophilus* expressed as CFU and as log of colony count when compared to groups containing uncoated wires. It was  $836.60 \pm 48.97$  CFU in the case of uncoated stainless steel whereas it was  $220.90 \pm 30.73$  CFU for

silver-modified stainless steel,  $748.90 \pm 35.64$  CFU for uncoated NiTi, and  $203.20 \pm 41.94$  CFU for surface-modified NiTi. It was concluded that Surface modification of orthodontic wires with silver can be used to prevent the accumulation of dental plaque and the development of dental caries during orthodontic treatment.

**Valiollah Arash et al<sup>53</sup>** in the year 2015 conducted a study to measure frictional resistance between silver coated brackets and different types of arch wires, and shear bond strength of these brackets to the tooth. In an experimental clinical research 28 orthodontic brackets (standard, 22 slots) were coated with silver ions using electroplate method. Six brackets (coated: 3, uncoated: 3) were evaluated with Scanning Electron Microscopy and Atomic Force Microscopy. The amount of friction in 15 coated brackets was measured with three different kinds of arch wires (0.019\_0.025-in stainless steel [SS], 0.018-in stainless steel [SS], 0.018-in Nickel-Titanium [Ni-Ti]) and compared with 15 uncoated steel brackets. In addition, shear bond strength values were compared between 10 brackets with silver coating and 10 regular brackets. Universal testing machine was used to measure shear bond strength and the amount of friction between the wires and brackets. SPSS 18 was used for data analysis with t-test. SEM and AFM results showed deposition of a uniform layer of silver measuring 8–10 nm in thickness on bracket surfaces. Silver coating led to higher frictional forces in all the three types of arch wires, which was statistically significant in 0.019\_0.025-in SS and 0.018-in Ni-Ti, but it did not change the shear bond strength significantly. Silver coating with electroplating method did not affect the bond strength of the bracket to enamel.

**Benjamin M. Geilich et al<sup>17</sup>** in the year 2015 conducted a study to explore the development and optimization of a polymersome nanocarrier formed from a biodegradable diblock copolymer to overcome bacterial antibiotic resistance. Here, polymersomes were synthesized containing silver nanoparticles embedded in the hydrophobic compartment, and ampicillin in the hydrophilic compartment. Results showed for the first time that these silver nanoparticle-embedded polymersomes (AgPs) inhibited the growth of *Escherichia coli* transformed with a gene for ampicillin resistance (*bla*) in a dose-dependent fashion. Free ampicillin, AgPs without ampicillin, and ampicillin polymersomes without silver nanoparticles had no effect on bacterial growth. The relationship between the silver nanoparticles and ampicillin was determined to be synergistic and produced complete growth inhibition at a silver-to-ampicillin ratio of 1: 0.64. In this manner, this study introduced a novel nanomaterial that can effectively treat problematic, antibiotic-resistant infections in an improved capacity which should be further examined for a wide range of medical applications.

**Sodagar A et al<sup>54</sup>** in the year 2016 conducted study to evaluate the antibacterial properties of a conventional orthodontic adhesive containing three different concentrations of silver/hydroxyapatite nanoparticles. One hundred and sixty-two Transbond XT composite discs containing 0, 1, 5, and 10 % silver/hydroxyapatite nanoparticles were prepared and sterilized. Antibacterial properties of these composite groups against *S.mutans*, *L.acidophilus*, and *Streptococcus sanguinis* were investigated using three different antimicrobial tests. Disk agar diffusion test was performed to assess the diffusion of antibacterial agent on brain heart infusion agar plate by measuring bacterial

growth inhibition zones. Biofilm inhibition test showed the antibacterial capacity of composite discs against resistant bacterial biofilms. Antimicrobial activity of eluted components from composite discs was investigated by comparing the viable counts of bacteria after 3, 15, and 30 days. Results showed that composite discs containing 5 and 10 % silver/hydroxyapatite nanoparticles were capable of producing growth inhibition zones for all bacterial types. All of the study groups showed reduced viable bacterial count in comparison to the control group. Antimicrobial activity of eluted components from composite discs was immensely diverse based on the bacterial type and the concentration of nanoparticles. It was concluded that Transbond xt composite discs containing 5 and 10 % silver/hydroxyapatite nanoparticles will produce bacterial growth inhibition zones and show antibacterial properties against biofilms.

**Ahmad Sodagar et al<sup>55</sup>** in the year 2016 conducted a study to assess the effects of adding nano-titanium dioxide and nano-silicon dioxide and their mixture to poly methyl methacrylate to induce antimicrobial activity in acrylic resins. Acrylic specimens in size of 20 mm × 20 mm × 1 mm of 0.5% and 1% of nano-TiO<sub>2</sub> (21 nm) and nano-SiO<sub>2</sub> (20 nm) and their mixture (TiO<sub>2</sub>/SiO<sub>2</sub> nanoparticles) (1:1 w/w) were prepared from the mixture of acrylic liquid containing nanoparticles and acrylic powder. To obtain 0.5% and 1% concentration, 0.02 g and 0.04 g of the nanoparticles was added to each milliliter of the acrylic monomer, respectively. Antimicrobial properties of six specimens of these preparations, as prepared, were assessed against planktonic *L. acidophilus* and *S. mutans* at 0, 15, 30, 45, 60, 75, and 90 min follow-up by broth dilution assay. The specimens of each group were divided into three subgroups: Dark,

daylight, or ultraviolet A (UVA). The percent of bacterial reduction was found out from the counts taken at each time point. Results showed that exposure to PMMA containing the nanoparticles reduced the bacterial count by 3.2–99%, depending on the nanoparticles, bacterial types, and light conditions. Planktonic cultures of *S. mutans* and *L. acidophilus* exposed to PMMA containing 1% of TiO<sub>2</sub>/SiO<sub>2</sub> nanoparticles showed a significant decrease (98% and 99%, respectively) in a time-dependent manner under UVA. The *S. mutans* and *L. acidophilus* counts did not significantly decrease in PMMA containing 0.5% nano-TiO<sub>2</sub> and PMMA containing 0.5% nano-SiO<sub>2</sub> in the dark. No statistically significant reduction was observed in the counts of *S. mutans* and *L. acidophilus* in PMMA without the nanoparticles exposed to UVA. It was concluded that PMMA resins incorporated with TiO<sub>2</sub>/SiO<sub>2</sub> nanoparticles showed strong antimicrobial activity against the cariogenic bacteria.

## **MATERIALS AND METHODS**

### **MATERIALS**

#### **Orthodontic materials:**

- Stainless Steel MBT .022" Slot pre adjusted edgewise appliance brackets (3M Gemini).

#### **Nano laboratory materials:**

- Planar magnetron sputtering unit (Nano sensor laboratory- PSG institute of advanced studies, Coimbatore)
- Scanning electron microscope (Mechanical Dept, Anna university, Chennai).

#### **Microbiological Laboratory materials:**

- Bacterial strains (Hi tech lab, Chennai)
- MRS broth (Hi tech lab, Chennai)
- BHI broth (Hi tech lab, Chennai)
- Petri dishes (Hi tech lab, Chennai)
- MRS agar plates (Hi tech lab, Chennai)
- BHI agar plates (Hi tech lab, Chennai)
- Anaerobic chamber (Hi tech lab, Chennai)
- Manual colony counter (Hi tech lab, Chennai)
- Incubator (Hi tech lab, Chennai)
- Spectrophotometer (Hi tech lab, Chennai)

### **METHOD**

This study was done on 120 specimens of stainless steel orthodontic brackets. The specimens were divided into six test groups. Each group consisted of 20 specimens.

## **STUDY DESIGN**

Study was allocated into 6 groups (experimental study)

- 20 brackets in each control group (20 x3= 60)
- 20 brackets in each experimental group (20 x3= 60)

### **Groups**

**Group 1A**-Control group –consist of 20 uncoated SS brackets in culture media with *S.mutans*.

**Group 1B**-Experimental group-consist of 20 surface coated SS brackets in culture media with *S.mutans*.

**Group 2A**-Control group –consist of 20 uncoated SS bracket in culture media with *L.acidophilus*.

**Group 2B**-Experimental group -consist of 20 surface coated SS brackets in culture media with *L.acidophilus*.

**Group 3A**-Control group –consist of 20 uncoated SS brackets in culture media with *P.gingivalis*.

**Group3B**-Experimental group –consist of 20 surface coated SS brackets in culture media with *P.gingivalis*.

### **Bacterial strains**

**L. acidophilus** (MTCC 447 ) Lactobacilli were inoculated into 5 ml of MRS broth and were incubated for 24 h at 37 °C.

**Strep. Mutans** (MTCC 890) were inoculated in 5 ml of a BHI and incubated for 24hours at 37°C.

**P. gingivalis** (ATCC 33277) The BHI broth containing 0.1% vitamin K1 and 1% hemin was used to cultivate *P. gingivalis*. *P. gingivalis* was grown at 37\_C; in an



anaerobic chamber with 85% nitrogen, 10% hydrogen, and 5% carbon dioxide (CO<sub>2</sub>) mixed gas.

### **Preparation of silver-coated orthodontic Brackets**

Surface modification of stainless steel orthodontic brackets with silver (Nano sensor laboratory , PSG institute of advanced studies ,Coimbatore) oxide was carried out by Magnetron sputtering method.

Sputtering process remove surface atoms or molecular fragments from a solid cathode (target) by bombarding it with positive ions from an inert gas (argon) discharge, and deposit them on the nearby substrate to form a thin film. Substrates are placed in a vacuum chamber and are pumped down to a prescribed process pressure. Sputtering starts when a negative charge is applied to the target material, causing a plasma or glow discharge. Positively charged gas ions generated in the plasma region are attracted to the negatively biased target plate at a very high rate of speed. This collision creates a momentum transfer and ejects atomically sized particles from the target. These particles are deposited as a thin film onto the surface of the substrates.

In this study, sputtering was carried out on stainless steel orthodontic brackets (substrate) using silver (Ag) as the target. A plasma generated inside the vacuumized chamber ejected surface atoms from the silver target, which were sputtered onto the stainless steel brackets (substrate). The distance between the substrate and the target was kept constant at 7 cm, and sputtering was conducted for a period of 10 minutes. All brackets were sputtered at the same time to achieve a thin and uniform coating of silver.

### **Analysis by scanning electron microscope (SEM)**

The surface morphology of the silver thin film was investigated with a scanning electron microscope (Mechanical Dept, Anna University, Chennai)

### **Antibacterial Activity Assay of Orthodontic Brackets**

**S.mutans-** S. mutans culture broth was diluted with BHI broth to make an optical density of 1.0 at 660 nm. Around 10 micro litre of the diluted bacterial suspension was transferred onto test tubes containing silver coated and uncoated stainless steel brackets. These tubes were incubated inside the laminar air flow chamber. After incubation, 100 ml of the bacterial suspension was serially diluted and plated onto BHI agar plates. Antibacterial activity was described as the survival rate by colony-forming units (CFUs) for S.Mutans using manual colony counter(Hi media laboratories)

**Lactobacilli-** lactobacillus culture broth was diluted with MRS broth to achieve an optical density of 1.0 at 660 nm. Ten micro liters of the diluted bacterial suspension was transferred onto test tubes containing uncoated and silver-coated stainless steel brackets. These tubes were incubated inside the laminar air flow chamber. After incubation, 100 ml of the bacterial suspension was serially diluted and plated onto MRS agar plates. Antibacterial activity was described as the survival rate by colony-forming units (CFUs).

**P.Gingivalis-** P. gingivalis culture broth was diluted with BHI broth to make an optical density of 1.0 at 660 nm.10micro litre of the diluted bacterial suspension was transferred onto test tubes containing either the uncoated stainless steel brackets or silver coated stainless steel brackets. These tubes were incubated

inside the anaerobic chamber. *P.gingivalis* is difficult to culture, Hence Antibacterial activity of the surface-modified orthodontic brackets was demonstrated by spectrophotometry. Reduction in optical density was measured. The mechanism of this method is based on the turbidity of the culture media for the evaluation of antibacterial properties of the materials containing antibacterial particles.

## **COLOUR PLATES**

**FIG.1.SHOWS UNCOATED ORTHODONTIC  
STAINLESS STEEL BRACKETS**



**FIG.2.SHOWS SILVER COATED ORTHODONTIC  
STAINLESS STEEL BRACKETS**



**FIG.3.SHOWS MAGNETRON SPUTTERING UNIT**



**FIG.4.SHOWS SILVER COATING PROCESS**



**FIG.5.SHOWS SCANNING ELECTRON MICROSCOPE**

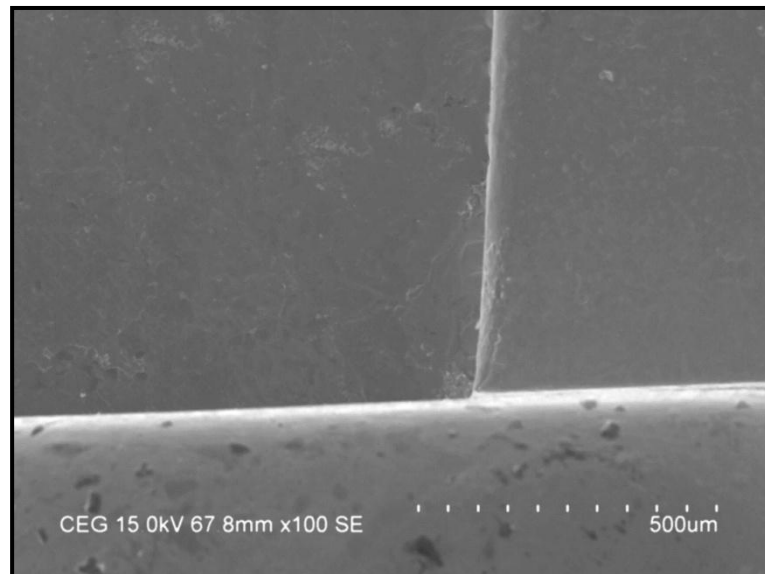


**FIG.6.SHOWS SCANNING ELECTRON MICROSCOPIC PROCEDURE**





**FIG.7. SHOWS SCANNING ELECTRON MICROSCOPIC  
FEATURE OF SILVER COATED SS BRACKET**



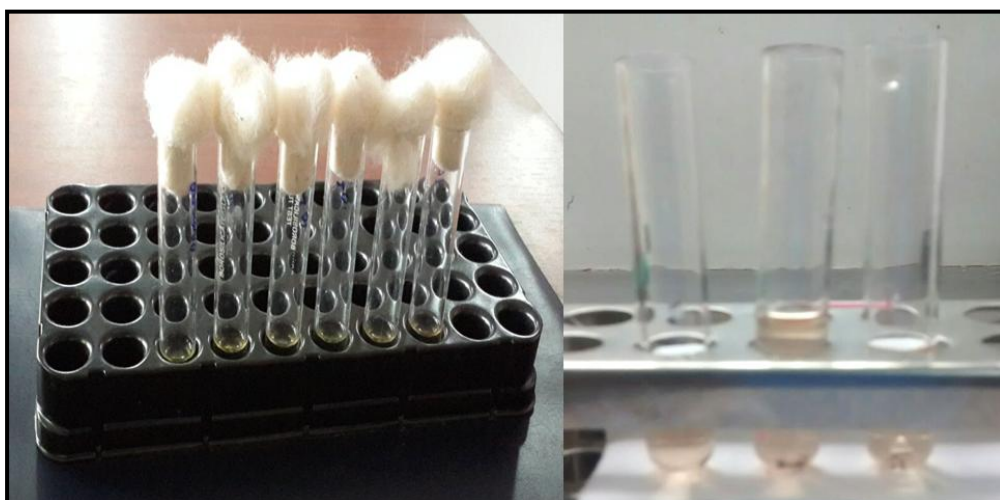
**FIG.8. SHOWS BACTERIAL STRAINS**



**FIG.9.SHOWS TEST TUBES CONTAINING MRS BROTH FOR  
S.MUTANS**

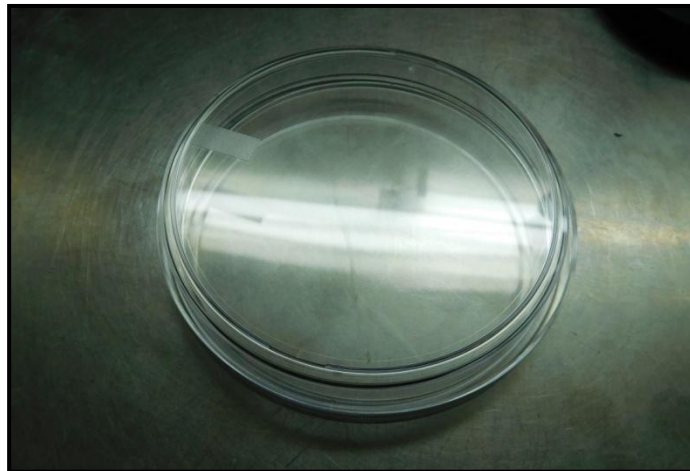


**FIG10.SHOWS TEST TUBES CONTAINING BHI BROTH FOR  
L.ACIDOPHILUS AND P.GINGIVALIS**





**FIG.11.SHOWS PETRI DISH**



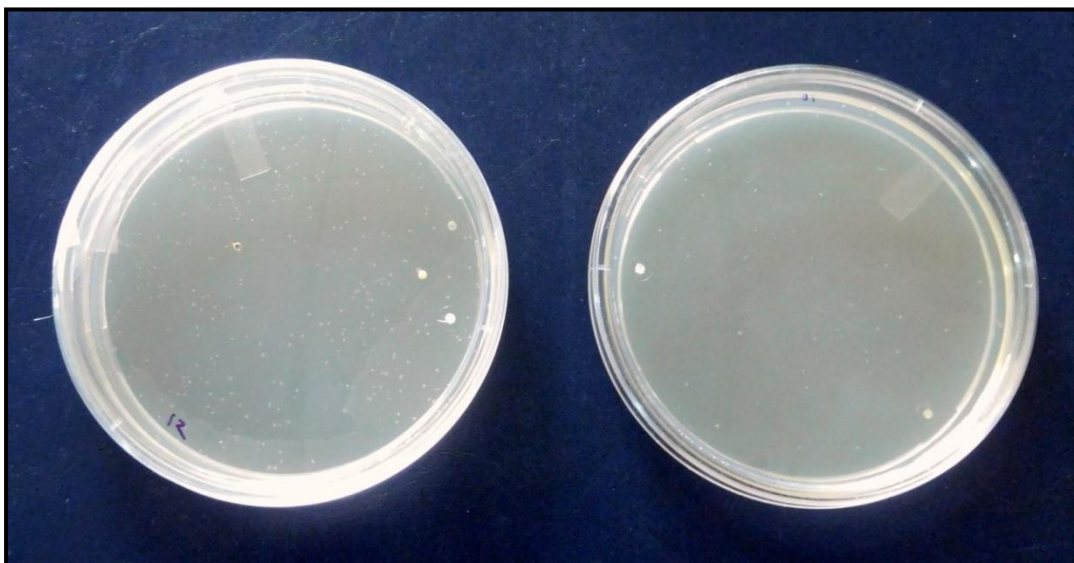
**FIG.12. SHOWS INCUBATOR**



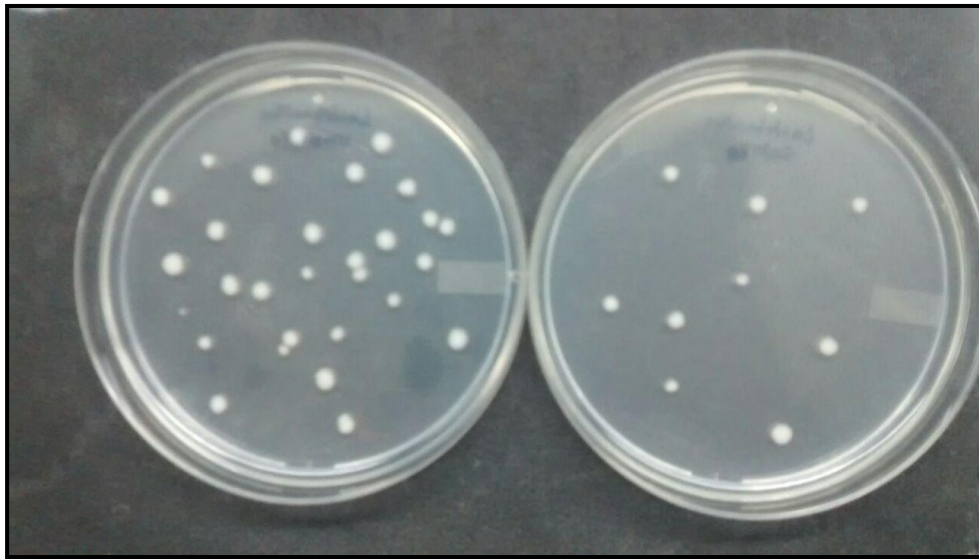
**FIG.13. SHOWS ANAEROBIC JAR**



**FIG.14. SHOWS PETRI DISHES CONTAINING S.MUTANS  
CONTROL AND EXPERIMENTAL GROUP**



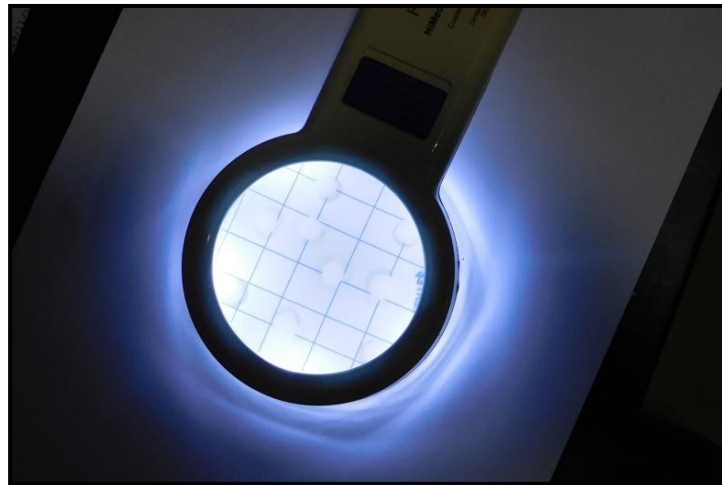
**FIG.15. SHOWS PETRI DISHES CONTAINING L.ACIDOPHILUS  
CONTROL AND EXPERIMENTAL GROUP**



**FIG.16. SHOWS MANUAL COLONY COUNTER**



**FIG.17. MANUAL COLONY COUNTER SHOWING  
BACTERIAL COLONIES**



**FIG.18. SPECTROPHOTOMETER SHOWING  
INITIAL OPTICAL DENSITY**



**FIG.19. SHOWS OPTICAL DENSITY FOR CONTROL AND TEST GROUP OF P.GINGIVALIS**



## **RESULTS**

### **STATISTICAL ANALYSIS**

The collected data was subjected to statistical analysis using SPSS version 17. The data was assessed for normality by Shapiro-Wilks test. Based on the distribution of data, the appropriate statistical test was used. Descriptive statistics were obtained for each group. The mean CFU were compared between uncoated and coated bracket group using unpaired t-test. Mann-Whitney U Test was used to compare reduction in optical density between groups.

The following parameters were analyzed:

1. Comparison 1: comparison of antimicrobial activity of silver coated stainless steel brackets and uncoated brackets against *S.mutans*
2. Comparison 2: comparison of antimicrobial activity of silver coated stainless steel brackets and uncoated brackets against *L.acidophilus*
3. Comparison 3: comparison of antimicrobial activity of silver coated stainless steel brackets and uncoated brackets against *P.gingivalis*
4. Comparison 4: comparison of antimicrobial activity of silver coated stainless steel brackets against *S.mutans* and *L.acidophilus*.

### **Antibacterial activity of surface-modified orthodontic brackets on *Strep.mutans***

Survival rate of the bacterial cells is calculated in terms of CFUs. The survival rate of *S.mutans* was  $77.85 \pm 12.893$  in control group. The survival rate of *S.mutans* in experimental group was  $53.60 \pm 10.990$ . P value was 0.000 ( $<0.005$ ). Log of colony count of uncoated brackets was  $1.8854 \pm 0.07347$ . Log of colony count of silver coated brackets was  $1.7203 \pm 0.090$ . P value for log of colony count was 0.000



(<0.005). Thus, the group containing surface-modified brackets showed statistically significant decrease in the survival rate of *S.mutans* when compared to uncoated group.

#### **Antibacterial activity of surface-modified orthodontic brackets on *L.acidophilus***

Survival rate of the bacterial cells is calculated in terms of CFUs. The survival rate of *L.acidophilus* was  $45.35 \pm 12.304$  in control group. The survival rate of *L.acidophilus* in experimental group was  $36.65 \pm 11.717$ . P value was 0.028 (<0.005). Log of colony count of uncoated brackets was  $1.6382 \pm 0.13663$ . Log of colony count of silver coated brackets was  $1.5355 \pm 0.1777$ . P value for log of colony count was 0.030 (<0.005). Thus, the groups containing surface-modified brackets showed statistically significant decrease in the survival rate of *L.acidophilus* when compared to groups containing uncoated stainless steel brackets.

#### **Antibacterial activity of surface-modified orthodontic brackets on *P.gingivalis***

Antibacterial activity of silver coated stainless steel brackets against *p.gingivalis* was demonstrated by spectrophotometry. Reduction in optical density was measured. Initial optical density of standard medium was 0.3. Optical density for uncoated orthodontic brackets against *P.gingivalis* is  $1.06 \pm .027$ . Optical density for silver coated orthodontic brackets against *P.gingivalis* is  $0.75 \pm .029$ . Optical density was reduced in coated group compared to uncoated group. Mann-Whitney U Test was used to compare reduction in optical density between groups. P value was < 0.05. As the bacterial count decreases optical density also decreases. Thus Antibacterial activity of surface-modified orthodontic brackets on *P.gingivalis* is statistically significant than uncoated brackets.

## COMPARISON BETWEEN SILVER COATED GROUPS AGAINST S.MUTANS AND L.ACIDOPHILUS

Unpaired student t test was used to compare the silver coated stainless steel orthodontic brackets against s.mutans and L.acidophilus. The survival rate of S.mutans in experimental group was  $53.60 \pm 10.990$ . The survival rate of L.acidophilus in experimental group was  $36.65 \pm 11.717$ . P value was 0.000 ( $<0.005$ ).

Thus antibacterial activity of silver coated stainless steel orthodontic brackets is more against S.mutans than L.acidophilus and this difference was statistically significant.

## TABLES AND CHARTS

**TABLE 1. GROUP STATISTICS FOR COLONY COUNT**

Bracket Group		N	Mean	Std. Deviation	Std. Error Mean
S.mutans (CFU)	<b>1A</b>	20	77.85	12.893	2.883
	<b>1B</b>	20	53.60	10.990	2.457
L.acidophilus (CFU)	<b>2A</b>	20	45.35	12.304	2.751
	<b>2B</b>	20	36.65	11.717	2.620
P.gingivalis (Reduction in Optical density)	<b>3A</b>	20	1.06	.119	.027
	<b>3B</b>	20	.75	.128	.029



**TABLE 2. TEST STATISTICS<sup>b</sup> FOR COLONY COUNT**

Tests	Reduction in OD-P.gingivalis
Mann-Whitney U	15.000
Wilcoxon W	225.000
Z	-5.091
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000 <sup>a</sup>

a. Not corrected for Ties

b. Grouping Variable: Bracket group

**TABLE 3. UNPAIRED STUDENT T TEST (PARAMETRIC TEST)  
FOR COMPARING ANTIBACTERIAL PROPERTY OF COATED AND  
UNCOATED BRACKETS AGAINST S.MUTANS AND L.ACIDOPHILUS**

Groups	N	Minimum	Maximum	Mean		Std. Deviation	95% Confidence Interval of the Difference		P value
				Statistic	Std. Error		Lower	Upper	
Group 1A	20	59	95	77.85	2.883	12.893	16.581	31.919	0.000*
Group 1B	20	35	75	53.60	2.457	10.990			
Group2A	20	20	65	45.35	2.751	12.304	1.009	16.391	0.028*
Group 2B	20	10	55	36.65	2.620	11.717			

\*Pvalue < 0.05 (significant)

**TABLE 4. MANN-WHITNEY U TEST (NON PARAMETRIC TEST) FOR COMPARING ANTIBACTERIAL PROPERTY OF COATED AND UNCOATED BRACKETS AGAINST P.GINGIVALIS**

Groups	N	Mean	Std. Deviation	Median	Interquartile range	P value
Group 3A	20	1.06	.027	0.336	0.041	0.000*
Group-3B	20	.75	.029	0.391	0.194	

\*Pvalue < 0.05 (significant)

**TABLE 5. UNPAIRED STUDENT T TEST (PARAMETRIC TEST) FOR COMPARING ANTIBACTERIAL PROPERTY OF COATED BRACKETS AGAINST S.MUTANS AND L.ACIDOPHILUS**

Bracket Groups	N	Minimum	Maximum	Mean		Std. Deviation	95% Confidence Interval of the Difference		P value
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Lower	Upper	
Group 1B	20	35	75	53.60	2.457	10.990	9.678	24.222	0.000*
Group 2B	20	10	55	36.65	2.620	11.71			

\*P value < 0.05 (significant)

**TABLE 6. GROUP STATISTICS FOR LOGARITHM OF COLONY COUNT**

<b>Bracket_ group</b>	<b>N</b>	<b>Mean</b>	<b>Std. Deviation</b>	<b>Std. Error Mean</b>
Group 1A	20	1.8854	.07347	.01643
Group 1B	20	1.7203	.09097	.02034
Group2A	20	1.6382	.13663	.03055
Group 2B	20	1.5355	.17771	.03974

**TABLE 7. TEST STATISTICS<sup>b</sup> FOR LOGARITHM OF COLONY COUNT**

<b>Tests</b>	<b>S.mutans Group</b>	<b>L.acidophilus Group</b>
Mann-Whitney U	29.000	120.000
Wilcoxon W	239.000	330.000
Z	-4.631	-2.170
Asymp. Sig. (2-tailed)	.000	.030
Exact Sig. [2*(1-tailed Sig.)]	.000 <sup>a</sup>	.030 <sup>a</sup>

a. Not corrected for ties.

b. Grouping Variable: Bracket group

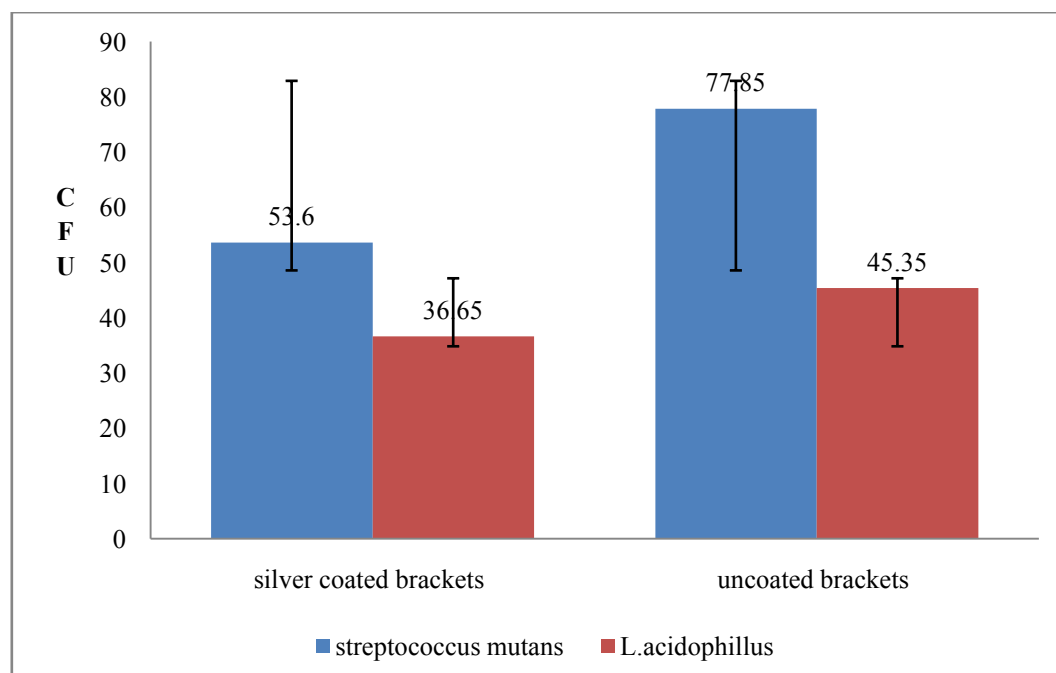
**TABLE 8. MANN-WHITNEY U TEST (NON PARAMETRIC TEST) FOR COMPARISON OF LOGARITHM OF COLONY COUNT BETWEEN COATED AND UNCOATED BRACKETS AGAINST S.MUTANS AND L.ACIDOPHILUS**

Groups	Mean	Std. Deviation	Median	Interquartile range	P value
Group 1A	1.8854	0.07347	1.89	0.14	0.000*
Group 1B	1.7203	0.09097	1.723	0.14	
Group 2A	1.6382	0.13663	1.6721	0.16	0.030*
Group 2B	1.5355	0.1777	1.5798	0.20	

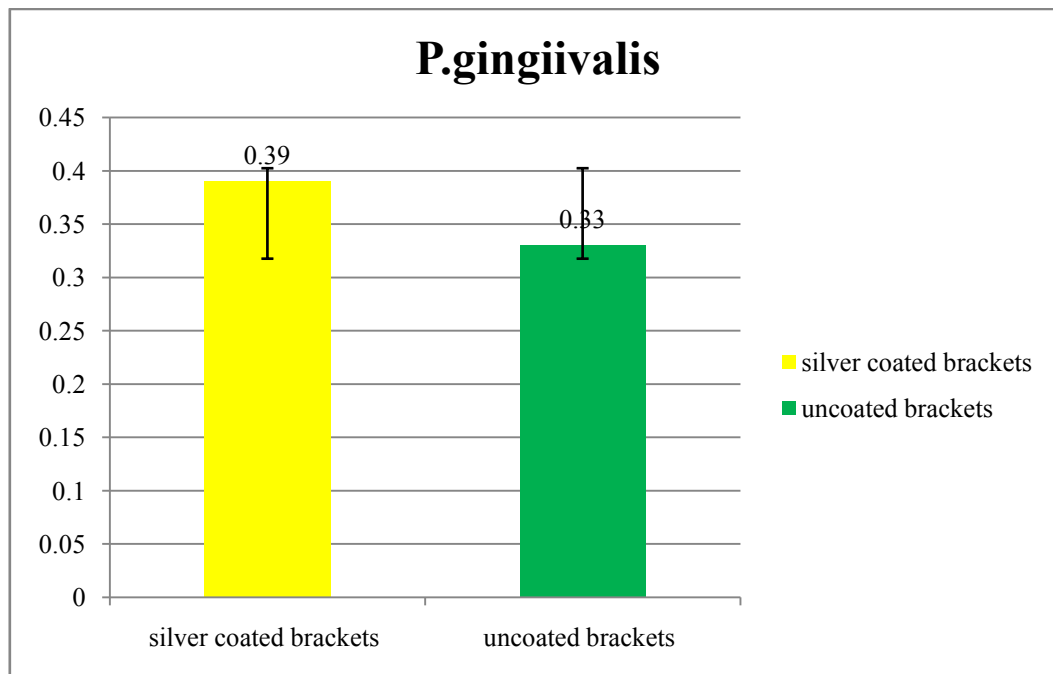
\*Pvalue < 0.05 (significant)

### CHARTS

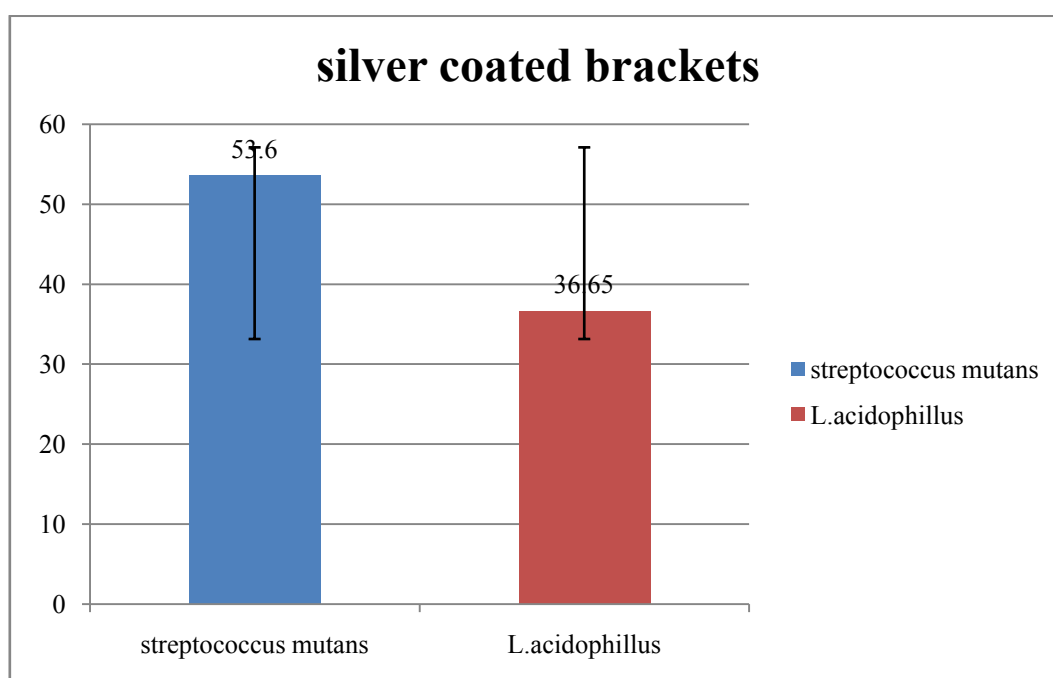
**CHART 1. SHOWING COMPARISON OF COLONY COUNTS BETWEEN COATED SS BRACKETS AND UNCOATED SS BRACKETS AGAINST S.MUTANS AND L.ACIDOPHILUS**



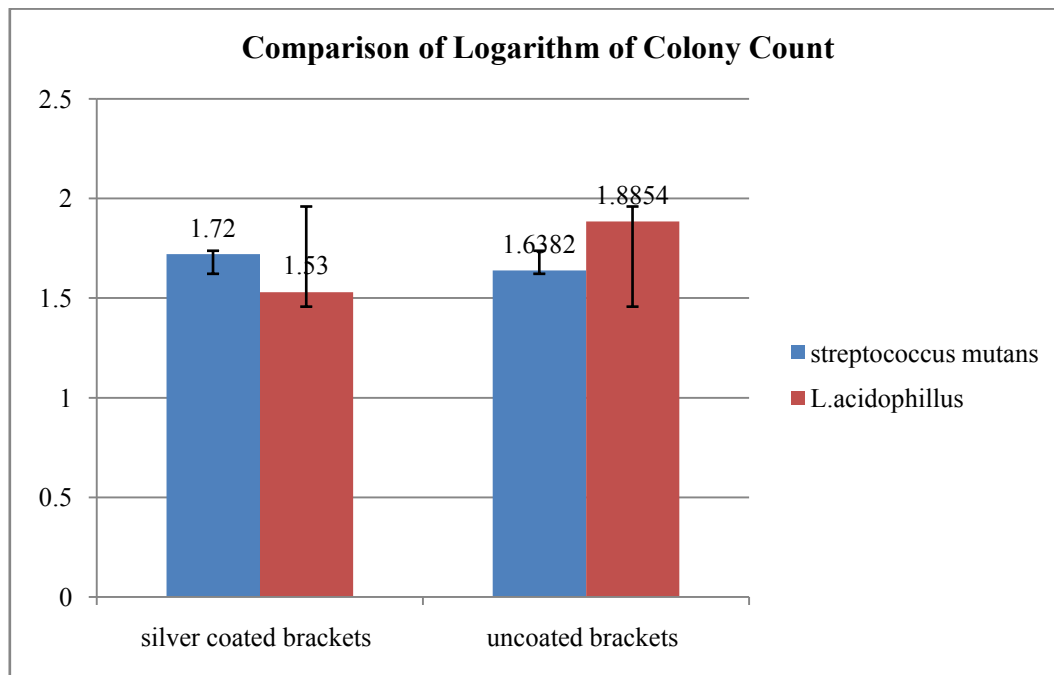
**CHART 2. SHOWING COMPARISON OF COLONY COUNTS BETWEEN SILVER COATED SS BRACKETS AND UNCOATED SS BRACKETS AGAINST P.GINGIVALIS**



**CHART 3. SHOWING COMPARISON OF COLONY COUNTS BETWEEN SILVER COATED SS BRACKETS AGAINST S.MUTANS AND L.ACIDOPHILUS**



**CHART 4. SHOWING COMPARISON OF LOGARITHM OF COLONY COUNTS BETWEEN COATED SS BRACKETS AND UNCOATED SS BRACKETS AGAINST S.MUTANS AND L.ACIDOPHILUS**



## DISCUSSION

Decalcification of the enamel surface adjacent to fixed orthodontic appliances is an important and prevalent iatrogenic effect of orthodontic therapy. The bonding of orthodontic appliances to teeth increases the number of plaque retention sites and, as a result, oral hygiene becomes more difficult. The low pH of plaque adjacent to orthodontic brackets hinders the remineralization process, and decalcification of enamel can occur<sup>56</sup>. As enamel translucency is directly related to the degree of mineralization<sup>57</sup>, initial enamel demineralization usually manifests itself clinically as a “white spot lesion” (WSL). The WSL has been defined as “subsurface enamel porosity from carious demineralization” that presents itself as “a milky white opacity when located on smooth surfaces.”<sup>57</sup>

Studies have shown that fixed orthodontic appliances induce a rapid increase in the volume of dental plaque and that such plaque has a lower pH than that in nonorthodontic patients<sup>58,59</sup>. Thus, the plaque-retentive properties of the fixed appliance predispose the patient to an increased cariogenic risk. Furthermore, there is a rapid shift in the composition of the bacterial flora of the plaque following the introduction of orthodontic appliances. More specifically, the levels of acidogenic bacteria, such as *S. mutans*, become significantly elevated in orthodontic patients. If these bacteria have an adequate supply of fermentable carbohydrates, acid by-products will be produced, lowering the pH of the plaque. As the pH drops below the threshold for remineralization, carious decalcification occurs<sup>60</sup>. With the progression of the caries, the number of streptococcus (Aerobic bacteria) decreases and that of lactobacillus (Anaerobic bacteria) increases. *L. acidophilus* is responsible for the progression of caries<sup>61,62</sup>. In the highly

cariogenic environment adjacent to orthodontic appliances or under loose bands, these lesions can rapidly progress. If left untreated, they may produce carious cavitations that will need an appropriate restoration. Thus, the prevention, of WSLs is crucial to prevent tooth decay as well as minimize tooth discoloration that could compromise the esthetics of the smile.

Biofilm present on the tooth, also causes gingival inflammation. Because of the unpredictable nature of the disease progression, all orthodontic patients with inflamed gingiva must be considered to be at risk for periodontal damage. The proximity of orthodontic brackets to the gingival sulcus, plaque accumulation, and the impediments they pose to oral hygiene habits further complicate the process of efficient salutary orthodontic care<sup>28-30,63</sup>. The effects seen clinically following the insertion of orthodontic appliances into the oral cavity can contribute to chronic infection, inflammatory hyperplasia, gingival recession, irreversible loss of attachment(permanent bone loss), and excessive accumulation of tissue, inhibiting complete extraction space closure.

Generally, as plaque accumulates, especially subgingivally, relatively benign Gram-positive cocci (commensal organisms) forms relent to the development of more pathogenic Gram-negative rods, spirochetes, and motile forms that define the pantheon of putative pathogens (periodontopathicbacteria), many of which are uncharacterized and not culturable for in vitro analysis<sup>64</sup>. Zachrisson reported that even after maintaining seemingly excellent oral hygiene, patients usually experience mild to moderate gingivitis within 1 to 2 months of appliance placement. These infective changes are generally quiescent, with no permanent damage introduced to tissues, except for 10% of adolescents, who



might show considerable irreversible periodontal attachment apparatus destruction<sup>28,29</sup>. *Porphyromonas gingivalis* (gram negative anaerobic bacilli) is a major pathogenic bacterium causing periodontitis.

Silver has an important microbial effect. The interaction of silver with thiol groups in enzymes and proteins plays an essential role in its antimicrobial action, although other cellular components, like hydrogen bonding, may also be involved. Silver has been proposed to act by binding to key functional groups of enzymes. It also causes the release of K<sup>+</sup> ions from bacterial plasma or cytoplasmic membrane, which is a site associated with many important bacterial enzymes, thus making it an efficient target site for silver action<sup>38</sup>.

Mi-Jin Chuna<sup>33</sup>, Choi<sup>38</sup>, Shaha<sup>42</sup> reported positive results in surface modification of stainless steel orthodontic wires and brackets with photocatalytic TiO<sub>2</sub> and TiAg (titanium silver). But there was discolouration of wires and brackets after TiO<sub>2</sub> coating and also there was loss of properties of NiTi wires on heating at 500 °C for 5 h. Mhaske et al<sup>27</sup> has reported no discoloration when NiTi and SS wires were coated with silver on investigating antibacterial property against *L.acidophilus*<sup>27</sup>. There is no clarity about antibacterial property of silver coated SS brackets against *S.mutans*, *L.acidophilus*, *P.gingivalis* in studies reported so far.

Size reduction of silver in nanoparticle form is an important condition for the effect of silver. Smaller size provides greater surface-to-volume ratio, leading to more close interaction with microbial membrane and larger surface area for antimicrobial activity<sup>27</sup>.

Hence this study was aimed to investigate the antibacterial property of silver coated SS brackets against *S.mutans*, *L.acidophilus*, *P.gingivalis*. Surface coating of silver can be obtained by different methods, like physical vapor deposition, electro deposition, electroless, and metallurgical<sup>65</sup>. According to Yamamoto<sup>66</sup> among all, physical vapor deposition exhibits a strong antimicrobial effect. So in this study, silver coating of stainless steel orthodontic brackets was carried out by magnetron sputtering method which is one of the physical vapor deposition methods.

Anti bacterial property of silver coated stainless steel bracket against *S.mutans*, *L.acidophilus* measured by counting colony forming unit (CFU).The number of bacteria was determined by conventional plate counting, which counts only culturable colonies in media. The survival rate of *S.mutans* was  $77.85 \pm 12.893$  in control group. where as survival rate of *S.mutans* in experimental group was  $53.60 \pm 10.990$ . P value was 0.000 ( $<0.005$ ). Thus, the group containing surface-modified brackets showed statistically significant decrease in the survival rate of *S.mutans* when compared to uncoated group. The survival rate of *L.acidophilus* was  $45.35 \pm 12.304$  in control group. The survival rate of *L.acidophilus* in experimental group was  $36.65 \pm 11.717$ . P value was 0.028 ( $<0.005$ ). Thus, the group containing surface-modified brackets showed statistically significant decrease in the survival rate of *L.acidophilus* when compared to groups containing uncoated stainless steel brackets. Anti bacterial property of silver coated stainless steel brackets against *P.gingivalis* was demonstrated by Spectrophotometry. Reduction in optical density was used to

assess the antibacterial activity. As the bacterial count decreases, optical density also decreases. Initial optical density of standard medium was 0.3. Optical density for uncoated orthodontic brackets against *P.gingivalis* is  $1.06 \pm .027$ . Optical density for silver coated orthodontic brackets against *P.gingivalis* is  $0.75 \pm .029$ . P value was 0.000 ( $<0.005$ ). Optical density of media containing silver coated brackets was significantly lower than uncoated brackets. This study findings showed that silver-coated brackets have effective antibacterial property against *S.mutans*, *L.acidophilus*, *P.gingivalis*.

Valiollah arash<sup>67</sup> observed higher frictional forces when NiTi and SS wires were coated with silver using electroplating method and but bond strength of bracket was not affected. Nevertheless silver coating on brackets is purely surface based and may be prone to wear during arch wire sliding. Thus, it is critical to assess the durability and sustainability of silver coatings under clinical situations in the oral environment. Hence it would be prudent to determine whether the thin coating of silver would alter its mechanical properties. However, the use of silver must be undertaken with caution, since the concentration-dependent toxicity has been demonstrated. Silver has not been mentioned in the list of the hazardous heavy metals to public health but still accumulation in the environment should be considered<sup>68</sup>. It was also proved that silver did not have cytotoxic or genotoxic effect.

It is essential to determine maximum lethal dose and the amount of silver necessary to carry out antibacterial properties before applying nanotechnology in orthodontics.

## **SUMMARY AND CONCLUSION**

White spot lesions and gingivitis are most common sequelae of fixed orthodontic appliance therapy. Studies have revealed that silver nanoparticles have antimicrobial property against bacteria, fungi and protozoa. This experimental study was carried out in 120 stainless steel orthodontic brackets, out of which 60 brackets were silver coated, 60 brackets were uncoated. These brackets were tested for its antimicrobial property against *S.mutans*, *L.acidophilus* and *P.gingivalis*. Antibacterial property of silver coated stainless steel brackets measured by counting colony forming unit (CFU). Antibacterial property of silver coated brackets against *P.gingivalis* demonstrated by reduction in optical density. These data were statistically analyzed and the following conclusions are arrived.

- Silver coated stainless steel orthodontic brackets shows more antibacterial activity against *S.mutans* compared to the control group.
- Silver coated stainless steel orthodontic brackets shows more antibacterial activity against *L.acidophilus* compared to the control group.
- Silver coated stainless steel orthodontic brackets shows more antibacterial activity against *P.gingivalis* compared to the control group.
- Silver coated stainless steel orthodontic brackets shows more antibacterial activity against *S.mutans* than *L.acidophilus* and this difference was statistically significant.
- Silver coating of stainless steel orthodontic brackets can be used to prevent development of dental plaque, thereby controlling the dental caries and periodontal disease.

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